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MAJOR ARTICLE

# Cytomegalovirus (CMV) Disease Despite Weekly Preemptive CMV Strategy for Recipients of Solid Organ and Hematopoietic Stem Cell Transplantation

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***Background.*** Transplant recipients presenting with cytomegalovirus (CMV) disease at the time of diagnosis of CMV DNAemia pose a challenge to a preemptive CMV management strategy. However, the rate and risk factors of such failure remain uncertain.

***Methods.*** Solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT) recipients with a first episode of CMV polymerase chain reaction (PCR) DNAemia within the first year posttransplantation were evaluated (n = 335). Patient records were reviewed for presence of CMV disease at the time of CMV DNAemia diagnosis. The distribution and prevalence of CMV disease were estimated, and the odds ratio (OR) of CMV disease was modeled using logistic regression.

***Results.*** The prevalence of CMV disease increased for both SOT and HSCT with increasing diagnostic CMV PCR load and with screening intervals >14 days. The only independent risk factor in multivariate analysis was increasing CMV DNAemia load of the diagnostic CMV PCR (OR = 6.16; 95% confidence interval, 2.09–18.11). Among recipients receiving weekly screening (n = 147), 16 (10.8%) had CMV disease at the time of diagnosis of CMV DNAemia (median DNAemia load 628 IU/mL; interquartile range, 432–1274); 93.8% of these cases were HSCT and lung transplant recipients.

***Conclusions.*** Despite application of weekly screening intervals, HSCT and lung transplant recipients in particular presented with CMV disease at the time of diagnosis of CMV DNAemia. Additional research to improve the management of patients at risk of presenting with CMV disease at low levels of CMV DNAemia and despite weekly screening is warranted.

**Keywords.** CMV disease; CMV DNAemia; CMV PCR; cytomegalovirus; transplant recipient.

Cytomegalovirus (CMV) DNAemia, detected in plasma with CMV polymerase chain reaction (PCR), is known to frequently complicate the course after solid organ transplantation (SOT) and human stem cell transplantations (HSCT) [1, 2]. Without intervention, CMV DNAemia may progress to CMV end organ disease [3]. The essence of the preemptive approach for prevention of CMV disease consists of CMV PCR measurements in whole blood or plasma at regular intervals after transplantation, to detect and, if above a predefined threshold, treat CMV DNAemia before it causes clinical disease [4]. HSCT recipients are usually managed solely preemptively, whereas SOT recipients usually receive universal prophylaxis with valganciclovir and/or preemptive follow-up after transplantation [2, 5]. Numerous previous studies have demonstrated that a high CMV DNAemia load in the blood compartment is among the most prominent risk factors for development of CMV disease [6–11]. To secure an early diagnosis while the DNAemia load remains low and the probability to prevent progression to CMV disease is high, current transplantation guidelines recommend weekly screening when applying a preemptive strategy for CMV prevention [2, 5]. The effectiveness of the preemptive strategy mainly depends on the meticulousness of, and adherence to, the screening program. However, some recipients may develop CMV end organ disease already at low or undetectable CMV PCR levels in the blood compartment; in particular, CMV pneumonia and CMV gastrointestinal (GI) disease have this tendency [12–16]. Recipients showing signs of CMV end organ disease at the diagnosis of CMV DNAemia implies failure of the preemptive strategy; however, the extent of this challenge in the setting of preemptive follow up remains to be fully elucidated. The aims of the present study were to assess the prevalence of CMV disease in a large, unselected cohort of transplant recipients at the time of diagnosis of the first episode

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of posttransplant CMV DNAemia in plasma, with special focus on potential CMV disease presenting at low levels of CMV DNAemia. Furthermore, we aimed to identify the main independent risk factor(s) of CMV disease at diagnosis of CMV DNAemia.

## METHODS

### Patients

Consecutive SOT (recipients of heart, lung, liver, kidney, and pancreas/kidney transplantation) and HSCT (recipients of myeloablative conditioning [MAC], nonmyeloablative conditioning [NMA], and umbilical cord blood [UCB]) recipients transplanted between January 1, 2010 to December 31, 2015 at Rigshospitalet, Copenhagen, Denmark were considered for inclusion in the study (n = 1673). Recipients with a known CMV immunoglobulin (Ig)G of the donor (D) and recipient (R) of either D+/R+, D−/R+, or D+/R− at transplantation (n = 1268) and who experienced a first episode of posttransplant CMV DNAemia (n = 335) in the blood compartment within the first year of transplantation were included in the present study.

The standard immunosuppressive regimens used at our center have been described previously [17]. The standard conditioning regimen for MAC consists of 12 grays of total body irradiation and a high dose (120 mg/kg) of cyclophosphamide.

### Management of CMV

The recipients were stratified according to subsequent risk of CMV DNAemia after transplantation as being either at high, intermediary, or low risk, based on the D/R CMV IgG serostatus at transplantation [18, 19]. The high-risk group comprised D+/R− SOT and D−/R+ HSCT recipients. The D+/R+ comprised the intermediary risk group for both types of transplantations, and the low-risk group comprised D−/R+ for SOT and D+/R− for HSCT.

CMV management in SOT and HSCT recipients at our center has been described previously [20]. In short, SOTs receive 3 months of prophylaxis and subsequent preemptive follow up. In case of side effects to valganciclovir prophylaxis, this is stopped and preemptive screening is commenced. Human stem cell transplantations are managed solely preemptively during the first year posttransplant.

CMV PCR monitoring was performed on plasma samples. COBAS AmpliPrep/COBAS TaqMan has been used since 2011, and before that the COBAS Amplicor kit was used [21]. In 2011, there was an overlap in the use of the 2 PCR platforms; during this time period, all tests were simultaneously tested on both platforms, determining a conversion factor between the COBAS Amplicor kit and the COBAS AmpliPrep/COBAS TaqMan at 1:1. The COBAS AmpliPrep/COBAS TaqMan conversion factor (1 copy/mL = 0.91 IU/mL) has been used to convert all CMV viral loads into IU/mL. The lower limit of detection was 273 IU/mL.

### CMV Infection and Disease Definitions

The present study focused exclusively on the time of diagnosis of the first episode of posttransplant CMV DNAemia detected in plasma using CMV PCR, defined as the date of the first of 2 consecutive plasma CMV PCR samples ≥273 IU/mL or of the first CMV PCR sample ≥2730 IU/mL.

The presence of CMV disease was assessed by a physician based on patient records at the time of diagnosis of CMV DNAemia, according to the present guidelines [3] (summarized in Supplementary Table 1). For CMV pneumonia and CMV GI disease, all potential copathogens detected ±7 days within the diagnostic CMV PCR were evaluated. For CMV GI disease in HSCT recipients, evidence of concurrent GI graft-versus-host disease (gvhd) was also evaluated in case of a concurrent biopsy.

The overall prevalence of CMV disease, as well as the prevalence after stratification for overall type of transplantation (SOT vs HSCT), was calculated. The prevalence of CMV disease was calculated for the respective types of transplantation in 4 different intervals depending on the DNAemia load in the diagnostic CMV PCR (DNAemia load [IU/mL]; <910, ≥910–2730, >2730–18 200, >18 200). Finally, the cohort was stratified into 4 groups depending on the time between the last negative CMV PCR sample and the consecutive diagnostic CMV PCR (<8 days, 8–14 days, >14–30 days, or >30 days), allowing for comparison of the median diagnostic CMV DNAemia load and prevalence of CMV disease between asymptomatic cases and cases with CMV disease. Univariate and multivariate logistic regression was used to determine the odds ratio (OR) for CMV disease at diagnosis of CMV DNAemia.

### Statistical Analyses

Standard descriptive statistics were used to compare baseline characteristics at the time of transplantation as well as differences in CMV DNAemia loads and proportions between the groups of recipients. Risk factors for CMV disease at the time of the diagnostic CMV PCR were explored using univariate and multivariate logistic regression adjusted for relevant confounders and tested for relevant interactions. All analyses were performed in SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC), and $P$ values ≤.05 were considered statistically significant.

## RESULTS

### Recipient Characteristics

Three hundred thirty-five recipients (178 SOT; 157 HSCT) were diagnosed with first-time CMV DNAemia in plasma with CMV PCR. Baseline demographics at the time of transplantation such as age and gender were comparable between SOT and HSCT recipients (Table 1). The SOT recipients had a higher proportion of low-risk patients developing CMV DNAemia compared with the HSCTs ($P$ = .002), but the distribution of high and intermediary risk profiles were comparable between the 2 groups of transplantation (Table 1).

**Table 1. Characteristics of Transplant Recipients With a First Episode of Cytomegalovirus DNAemia Detected in Plasma, Stratified for Type of Transplantation**

| Features of Included Recipients and the First Episode of CMV DNAemia | Solid Organ Recipients N = 178 | Bone Marrow Recipients N = 157 | *P* Value |
|---|---|---|---|
| Characteristics of Recipients | | | |
| Median age at transplantation (IQR) | 50 (38–59) | 48 (33–61) | .6 |
| Gender (% males) | 98 (55.06%) | 100 (63.69%) | .1 |
| Type of Transplantation (% of Transplantation Type) | | | |
| Kidney | 74 (41.57%) | N.A. | |
| Liver | 42 (23.59%) | N.A. | |
| Lung | 52 (29.21%) | N.A. | |
| Heart | 8 (4.49%) | N.A. | |
| Kidney/Pancreas | 2 (1.12%) | N.A. | |
| MAC | N.A. | 76 (48.41%) | |
| NMA | N.A. | 68 (43.31%) | |
| UCB | N.A. | 13 (8.28%) | |
| Risk of CMV Infection Associated With Donor and Recipient CMV IgG Serostatus[a] | | | |
| Proportion of recipients at high risk | 86 (48.31%) | 87 (55.41%) | .2 |
| Proportion of recipients at intermediary risk | 72 (40.45%) | 66 (42.04) | .8 |
| Proportion of recipients at low risk | 20 (11.24%) | 4 (2.55%) | .002 |
| Characteristics of the First Episode of CMV DNAemia | | | |
| Median time (days) from transplantation to CMV DNAemia (IQR) | 120 (63–148) | 48 (35–62) | <.0001 |
| Median time (days) from the last negative CMV PCR to the first positive CMV PCR of the CMV DNAemia (IQR) | 18 (7–28) | 7 (6–9) | <.0001 |
| Median DNAemia load (IU/mL) of the first positive CMV PCR sample of the CMV DNAemia episode (IQR) | 719 (282–2548) | 455 (273–910) | .0005 |

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G; IQR, interquartile range; MAC, myeloablative conditioning; N.A., not applicable; NMA, nonmyeloablative conditioning; PCR, polymerase chain reaction; UCB, umbilical cord blood.

[a]Risk of CMV DNAemia according to donor (D)/recipient (R) CMV IgG serostatus (±) at the time of transplantation. For solid organ recipients, high risk of CMV DNAemia is associated with D+/R−, whereas D−/R+ represent the high-risk group among hematopoietic stem cell recipients. For both types of transplantation, D+/R+ is associated with an intermediary risk of CMV infection. The low-risk group constitutes D−/R+ for solid organ recipients and D+/R− for hematopoietic stem cell recipients.

Of the 157 HSCT recipients, 137 were matched unrelated donors, and the remaining 20 were matched family (parent or sibling) donors.

***CMV DNAemia.***

Due to the administration of valganciclovir prophylaxis to the SOT population, CMV DNAemia developed later in SOT recipients compared with HSCT recipients ($P < .0001$) (Table 2). The median DNAemia load of the diagnostic CMV PCR was higher in SOT recipients compared with the HSCT recipients (diagnostic CMV PCR 719 IU/mL [interquartile range {IQR}, 282–2548] vs 455 IU/mL [IQR, 273–910]; $P = .0005$). The overall median time of days from the last negative CMV PCR to the first positive CMV PCR (eg, the screening interval) was significantly longer in SOT recipients than HSCT recipients ($P < .0001$) (Table 1).

***Overall CMV Disease at the Time of CMV DNAemia Diagnosis.***

Overall, 49 of 335 (14.6%; 95% confidence interval [CI], 10.8–18.4) of the included recipients had CMV disease at the time of diagnosis of the first episode of CMV DNAemia, corresponding to an overall 18.5% (95% CI, 12.8–24.2) of the SOT recipients and 10.2% (95% CI, 5.5–14.9) of the HSCT (Figure 1). The clinical manifestations detected in the cohort at the time of diagnosis of CMV DNAemia were CMV syndrome, CMV pneumonia, and CMV GI disease, and the prevalence and type of CMV disease differed between SOT and HSCT recipients (Figure 1 and Supplementary Table 2). Overall, there were 64 deaths during the first year of transplantation; of these, 13 occurred in patients who presented with CMV disease at CMV DNAemia diagnosis. There were 20 deaths occurring within 28 days after the last positive CMV PCR of the first CMV DNAemia episode; of these, 6 occurred in patients who presented with CMV disease at CMV DNAemia diagnosis.

***CMV Disease and CMV DNAemia Load.***

Cases with CMV disease had a significantly higher median DNAemia load compared with the asymptomatic episodes of CMV DNAemia (3913 IU/mL [IQR, 728–91 000] vs 478 IU/mL (IQR, 273–1001); $P < .0001$). Overall, the prevalence of CMV disease in SOT and HSCT stratified for the DNAemia load in the diagnostic CMV PCR increased with increasing load of DNAemia (Figure 2). However, for both main types of transplantations, CMV organ disease could be found at the time of diagnosis already at DNAemia levels <910 IU/mL, with a prevalence of 8.9% (95% CI, 3.4–14.4) for the SOT recipients and 5.1% (95% CI, 1.1–9.1) for the HSCT recipients (Figure 2). All of the SOT recipients with CMV disease at diagnostic CMV

**Table 2. Univariate and Multivariate Logistic Regression of Risk Factors for Cytomegalovirus Disease Among 335 Transplant Recipients With a First CMV DNAemia Detected in Plasma With CMV PCR**

| | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| Factors | OR | (95% CI) | P Value | OR | (95% CI) | *P* Value |
| Baseline Characteristics | | | | | | |
| Male gender | 1.00 | (0.54–1.86) | .99 | 0.85 | (0.41–1.78) | .67 |
| Age at transplantation | 1.04 | (0.87–1.24) | .66 | 1.02 | (0.82–1.27) | .85 |
| Year of Transplantation | | | | | | |
| 2010 | 1.93 | (0.71–5.21) | .20 | 1.18 | (0.35–4.02) | .79 |
| 2011 | 1.35 | (0.46–3.92) | .59 | 1.25 | (0.36–4.31) | .72 |
| 2012 | 1.10 | (0.44–2.80) | .83 | 0.93 | (0.32–2.71) | .89 |
| 2013 | 1.33 | (0.50–3.53) | .56 | 0.81 | (0.24–2.74) | .73 |
| 2014 | 0.65 | (0.23–1.83) | .42 | 0.77 | (0.24–2.44) | .66 |
| 2015 | | Ref. | | | Ref. | |
| Type of Transplantation | | | | | | |
| HSCT | | Ref. | | | Ref. | |
| SOT | 2.01 | (1.06–3.81) | .03 | 1.18 | (0.50–2.83) | .71 |
| Risk associated with CMV IgG serostatus[a] | | | | | | |
| High risk | | Ref. | | | Ref. | |
| Intermediary and low risk | 0.77 | (0.42–1.42) | .41 | 1.21 | (0.58–2.54) | .61 |
| CMV DNAemia Load of the First Positive CMV PCR Sample (IU/mL) | | | | | | |
| CMV DNAemia load <910 | | Ref. | | | Ref. | |
| CMV DNAemia load ≥910–2730 | 1.76 | (0.69–4.54) | .24 | 1.85 | (0.69–4.98) | .22 |
| CMV DNAemia load >2730–18 200 | 5.73 | (2.15–15.23) | .0005 | 6.16 | (2.09–18.11) | .001 |
| CMV DNAemia load >18 200 | 28.71 | (11.10–74.30) | <.0001 | 44.86 | (11.86–169.65) | <.0001 |
| Screening Interval[b] | | | | | | |
| <8 days | | Ref. | | | Ref. | |
| ≥8–14 days | 0.81 | (0.32–2.05) | .65 | 0.59 | (0.21–1.67) | .32 |
| >14–30 days | 2.30 | (1.08–4.91) | .03 | 0.72 | (0.25–2.13) | .56 |
| >30 days | 3.03 | (1.24–7.40) | .01 | 0.38 | (0.09–1.60) | .19 |

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation; IgG, immunoglobulin G; OR, odds ratio; PCR, polymerase chain reaction; Ref., reference; SOT, solid organ transplantation.

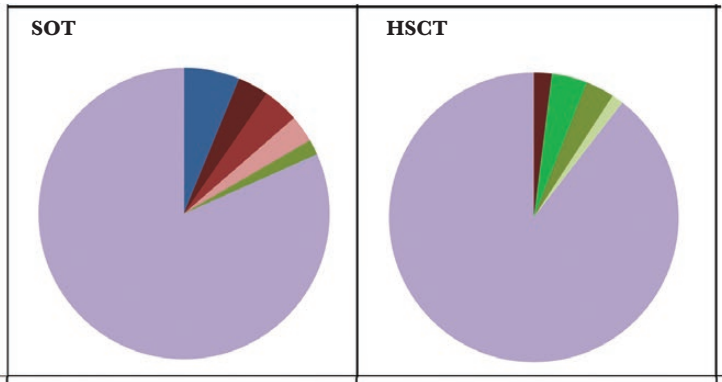
[a]Risk of CMV DNAemia according to donor (D)/recipient (R) CMV IgG serostatus (±) at the time of transplantation. For solid organ recipients, high risk of CMV DNAemia is associated with D+/R−, whereas D−/R+ represents the high-risk group among hematopoietic stem cell recipients. For both types of transplantation, D+/R+ is associated with an intermediary risk of CMV infection. The low-risk group constitutes D−/R+ for solid organ recipients and D+/R− for hematopoietic stem cell recipients.

[b]Based on the interval of days between the last negative CMV PCR and the diagnostic CMV PCR of the CMV DNAemia episode.

PCR levels <910 IU/mL were recipients of lung transplantation (1 probable GI disease; 8 cases of CMV pneumonia [3 proven, 4 probable, and 1 possible]); for HSCT recipients, the GI diseases present in this interval of CMV DNAemia were proven in 4 cases and probable in 2 cases. The DNAemia load of the diagnostic CMV PCR increased with longer screening interval among the symptomatic recipients (Figure 3). In comparison, this trend was less pronounced among the asymptomatic recipients. Furthermore, the symptomatic episodes of CMV DNAemia had significantly higher CMV DNAemia load regardless of screening interval. The proportion of CMV disease was 10.8% (95% CI, 5.8–15.8) and 9.0% (95% CI, 2.6–15.3), already at screening intervals <8 days and ≥8–14 days, respectively, and increased to 27.0 (95% CI, 12.7–41.3) in patients with screening intervals >30 days. Among the recipients constituting the 10.8% with CMV disease despite weekly screening, 15 of 16 (93.7%; 95% CI, 81.8–105.6) cases were organ disease (GI disease: 9 HSCT and 1 lung recipient; CMV pneumonia: 5 lung transplant recipients), with a median diagnostic DNAemia load at 628 IU/mL (IQR, 432–1274). More importantly, only 2 of these cases of organ disease were possible cases (1 lung transplant with pneumonia and 1 NMA recipient with GI disease). The one case with CMV syndrome was a CMV IgG D+/R− kidney recipient who was diagnosed with CMV DNAemia at 91 000 IU/mL 5 days after the last negative CMV PCR. In the multivariate logistic regression model, only CMV DNAemia load >2730 IU/mL remained an independent risk factor for CMV disease at the time of diagnosis of CMV DNAemia, and the OR increased with increasing diagnostic DNAemia load (Table 2). There were no interactions between the type of transplantation (SOT vs HSCT) and the viral load in the diagnostic CMV PCR.

## DISCUSSION

In this study, we found that despite application of the recommended weekly screening interval for preemptive screening, 10.8% of the patients had concomitant CMV disease at the time of CMV DNAemia diagnosis. This indicates that a subset of




| Type of clinical manifestation | SOT (n=178) | | HSCT (n=157) | | Colour coding |
|---|---|---|---|---|---|
| | % | [95% CI] | % | [95% CI] | |
| Asymptomatic CMV DNAemia | 81.5 | 75.8–87.2 | 89.8 | 85.1–94.5 | |
| Overall CMV disease | 18.5 | 12.8–24.2 | 10.2 | 5.5–14.9 | NA |
| Possible CMV GI disease | 0.06 | –0.3–0.4 | 1.3 | –0.5–3.1 | |
| Probable CMV GI disease | 1.7 | –0.2–3.6 | 3.2 | 0.4–6.0 | |
| Proven CMV GI disease | 0 | 0 | 3.8 | 0.8–6.8 | |
| Possible CMV pneumonia | 2.8 | 0.4–5.2 | 0 | 0 | |
| Probable CMV pneumonia | 3.9 | 1.1–6.7 | 0 | 0 | |
| Proven CMV pneumonia | 3.4 | 0.7–6.1 | 1.9 | –0.2–4.0 | |
| CMV syndrome | 6.2 | 2.7–9.7 | NA | NA | |

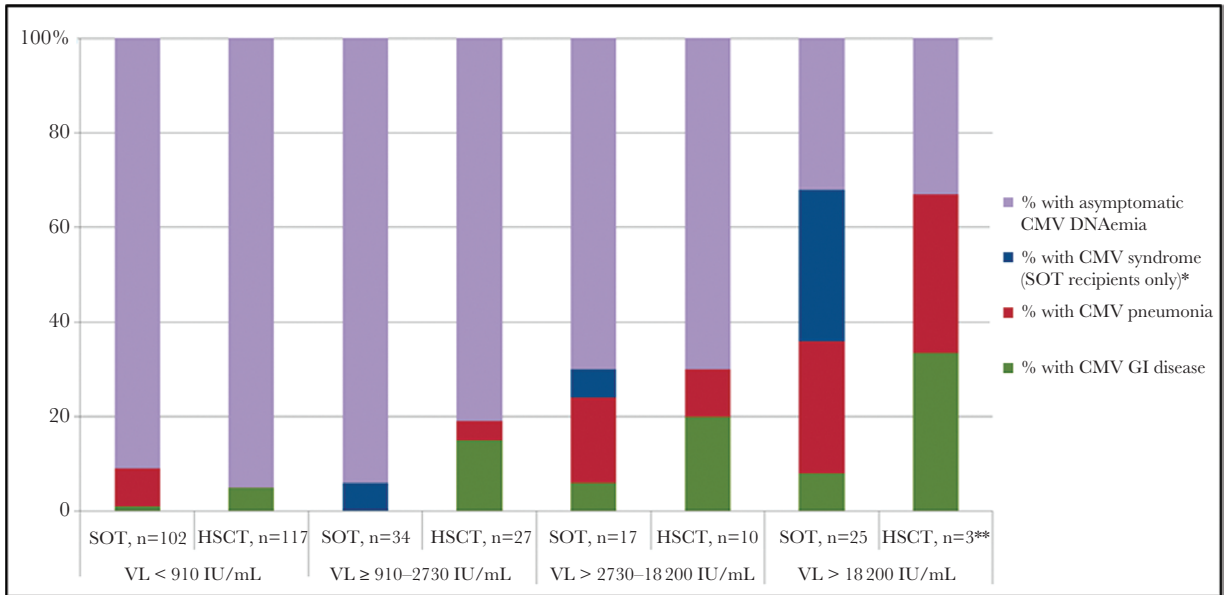
**Figure 1.** Prevalence and type of cytomegalovirus (CMV) disease at the first CMV DNAemia episode in solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT) recipients. No other types of CMV disease were observed. CI, confidence interval; GI, gastrointestinal; NA, not applicable.

transplant recipients is at risk of failing the preemptive strategy despite application of recommended screening intervals.

Several previous studies demonstrated that the CMV viral load can predict the clinical outcome of CMV infection in transplant recipients and that the risk of CMV disease increases with increasing CMV DNAemia load [7–11]. In line with this, the OR for CMV disease at the time of diagnosis of CMV DNAemia increased with increasing DNAemia load of the diagnostic CMV PCR in the present study (Table 2). However, the CMV DNAemia load was <910 IU/mL for the majority of the weekly screened recipients presenting with CMV disease concurrent with the CMV DNAemia diagnosis (Figure 3). The infectious phenotypes for CMV disease occurring at these low levels of CMV DNAemia were mainly confined to CMV GI disease in HSCT recipients and CMV pneumonia in lung transplant recipients.

Other studies have reported similar observations of CMV disease being compartmentalized in organs such as the lung and GI canal, with corresponding low or absent CMV DNAemia in the blood compartment [13–16, 22–24]. The concept of low or absent CMV DNAemia concurrent with CMV end organ disease poses a massive challenge to CMV screening strategies, because the success of screening and subsequent preemptive treatment relies on the hypothesis that CMV disease is precipitated by CMV DNAemia and that the risk is increasing with higher DNAemia loads [6]. Although there are studies focusing on the progression of CMV DNAemia to CMV disease on preemptive treatment [25], very few observations have focused on patients managed with preemptive screening yet failing already at the time of diagnosis of CMV DNAemia. Our results indicate that low diagnostic CMV DNAemia levels do not rule out CMV GI disease in HSCT recipients or CMV pneumonia in lung transplant recipients, and it adds further evidence to the current clinical practice that patients with symptoms of these phenotypes of organ invasive disease should be investigated regardless of plasma DNAemia load and, in particular, if present in any of these types of transplantation recipients.

Although the preemptive strategy has proven successful in many previous studies to prevent CMV disease [4, 26, 27], this observation also underlines the importance of finding alternatives to the solely preemptive strategy applied to HSCT recipients. There are several ongoing trials with newer drug compounds (such as letermovir for prophylaxis) that potentially could be administered to HSCT recipients [28–31], delaying CMV DNAemia to a potentially more stable period posttransplantation. On a similar note, 3 months of prophylaxis and subsequent screening for detection and diagnosis of CMV pneumonia might not be sufficient for the lung transplant recipients, reflecting the ongoing debate of duration of prophylaxis in this group of recipients [32, 33]. In fact, since this study,




| Intervals of DNAemia load at time of CMV DNAemia diagnosis | SOT | | HSCT | |
|---|---|---|---|---|
| | % | [95% CI] | % | [95% CI] |
| **VL <910 IU/mL** | | | | |
| **Asymptomatic CMV DNAemia** | 91.1 | 85.6–96.6 | 94.9 | 90.9–98.9 |
| **Overall CMV disease** | 8.9 | 3.4–14.4 | 5.1 | 1.1–9.1 |
| **VL ≥910–2730 IU/mL** | | | | |
| **Asymptomatic CMV DNAemia** | 94.1 | 86.2–102.0 | 81.5 | 66.9–96.1 |
| **Overall CMV disease** | 5.9 | −2.0–13.8 | 18.5 | 3.9–33.1 |
| **VL >2730–18 200 IU/mL** | | | | |
| **Asymptomatic CMV DNAemia** | 70.6 | 48.9–92.3 | 70.0 | 41.6–98.4 |
| **Overall CMV disease** | 29.4 | 7.7–51.1 | 30.0 | 1.6–58.4 |
| **VL >18 200 IU/mL** | | | | |
| **Asymptomatic CMV DNAemia** | 32.0 | 13.7–50.3 | 33.3** | −20.0–86.6 |
| **Overall CMV disease** | 68.0 | 49.7–86.3 | 66.7** | 13.4–120.0 |

**Figure 2.** Prevalence of cytomegalovirus (CMV) disease (by type) according to diagnostic DNAemia load at the first episode of CMV DNAemia in solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients. *Only applicable to SOT recipients. **Please note the low number of HSCT recipients in this interval of CMV DNAemia. CI, confidence interval; VL, viral load.

the valganciclovir prophylaxis has been prolonged for the lung transplant recipients in our cohort.

CMV GI disease and pneumonia are both complex diagnoses to establish. CMV infection has been associated with increased association with secondary bacterial and fungal infections and gvhd [15, 16, 22, 34]. For example, the clinical presentations of CMV GI disease and GI gvhd are very similar, and they are usually impossible to differentiate without an endoscopic investigation and biopsy [22, 35]. Thus, the presence of competing causes makes it difficult to discern the role of CMV as the culprit or opportunistic bystander. For this study, we have applied the new definitions on CMV disease in transplant recipients [3], and we have reported concurrent competing causes. Our data imply the necessity to continue to investigate all biopsies from the GI canal for CMV in HSCT recipients with GI symptoms, regardless of their concurrent CMV DNAemia levels. Likewise, CMV pneumonia should be suspected in lung transplant recipients with respiratory symptoms irrespective of plasma PCR results, and, if possible, bronchoalveolar lavage (BAL) CMV PCR should be performed [15].

There are some limitations in this study. The plasma CMV PCR was collected as a part of the routine prospective follow up, but the evaluation of the potential presence of CMV disease in the CMV DNAemia episodes were performed retrospectively

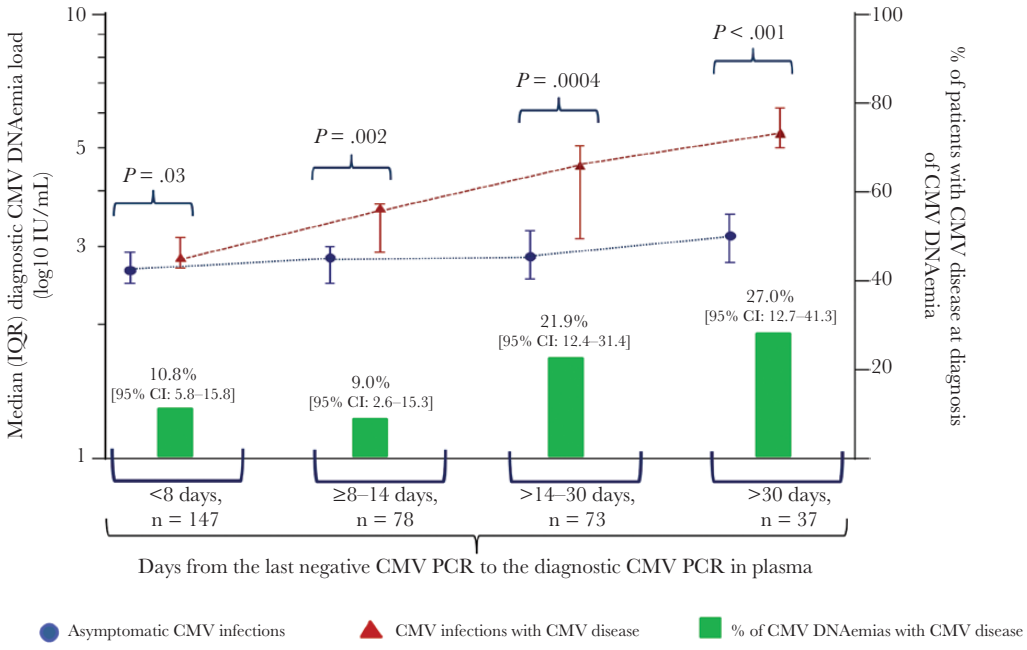



**Figure 3.** Cytomegalovirus (CMV) DNAemia load and disease at the time of CMV DNAemia diagnosis according to screening interval. The cohort was stratified depending on the number of days between the last negative CMV polymerase chain reaction (PCR) and the diagnostic CMV PCR of the CMV DNAemia episode. The median (interquartile range [IQR]) diagnostic DNAemia load of asymptomatic CMV DNAemia episodes (blue circles) and of the CMV disease episodes (red triangles) were calculated and compared for each screening interval. Furthermore, the prevalence of CMV disease (green bars) was calculated for each screening interval. CI, confidence interval.

from patient journal records. The CMV PCR results presented in the current study are transformed using the international standard introduced by the World Health Organization [36]; however, it is important to recall that some variability still remains despite this when interpreting the reported viral loads [37, 38]. Furthermore, the current observations were based on CMV PCR performed in plasma, and, as such, the results may not be directly transferable to other cohorts that, for example, use whole blood for CMV PCR screening [39]. Finally, the aim of this study was to evaluate the presence of CMV disease at the time of the diagnostic CMV PCR in plasma of the first episode of posttransplant CMV DNAemia. As such, we have not evaluated the negative CMV PCRs of our cohort for presence of CMV disease, and, as a consequence, there may be isolated cases of organ disease with concurrent negative plasma CMV PCR. In fact, we have recently investigated the diagnostic yield of BAL CMV PCR in lung transplant recipients, where we demonstrated the presence of proven and probable CMV pneumonia with concurrent negative plasma CMV PCRs [15]. However, a systematic review of all pathology data during CMV PCR-negative time periods remains to be performed before we can ascertain the number of biopsy proven cases of CMV organ disease, regardless of CMV PCR results.

## CONCLUSIONS

In conclusion, increasing viral load at the time of diagnosis of CMV DNAemia was the most prominent risk factor for CMV disease. However, a remarkably large proportion of the episodes with CMV disease occurred in recipients who were weekly screened and who were diagnosed at levels of CMV DNAemia <910 IU/mL. These cases consisted almost exclusively of HSCT and lung transplant recipients, who presented with CMV GI disease and CMV pneumonia. These observations underline the need for additional research to improve the understanding of the factors explaining the development of CMV disease at low CMV PCR viral loads, especially for CMV GI disease in HSCT recipients and CMV pneumonia in lung transplant recipients.

## Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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